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EFFECTS OF HYDRALAZINE ON CARDIAC RESPONSIVENESS TO ADRENERGIC AGONISTS IN STREPTOZOTOCIN-INDUCED DIABETIC RATS*

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Abstract: The present investigation was undertaken to study the effects of hydralazine treatment (50 mg/kg/day, p.o.) on methoxamine and isoproterenol-induced responses in cardiac preparations of control and streptozotocin (STZ)-induced diabetic rats. Triiodothyronine (T_3) and thyroxine (T_4) levels were found to be significantly decreased in diabetic rats and this decrease was prevented by hydralazine treatment. Methoxamine and isoproterenol produced a dose-dependent positive chronotropic and positive inotropic effect in right and left atrium respectively. These responses to methoxamine were significantly increased, whereas, those to isoproterenol were significantly decreased in preparations obtained from diabetic rats. Hydralazine treatment did not alter the isoproterenol-induced chronotropic effect in right atrium. However, it prevented the diabetes-induced increase in responsiveness to methoxamine in this preparation. Hydralazine increased significantly the inotropic response to methoxamine and isoproterenol in left atrium of control and diabetic rats. Both the pD_2 value and maximum response were increased. The studies indicates that hydralazine-induced alteraions in the responsiveness to methoxamine could partly be due to its ability to prevent diabetes-induced hypothyroidism. The effects of hydralazine on isoproterenol-induced responses appear to be independent of hypothyroidism, and some post-receptor mechanisms and metabolic derangements might be responsible for this effect.

Key words : streptozotocin, isoproterenol, left atrium, methoxamine, right atrium, hydralazine

INTRODUCTION

Rodrigues et al (1) reported that hydralazine not only prevents development of hypertension and hyperlipidaemia in diabetic rats but also improves cardiac function. While discussing the mechanisms involved in hydralazine-induced alterations in diabetic rats it was proposed that the failure of hydralazine-treated diabetic rats to show elevated serum lipids could be due to its direct effect on catecholamine synthesis and release. Furthermore, the observation that hydralazine prevents diabetes-induced hypothyroidism further strengthens this assumption (1). Both diabetes and hypothyroidism are known to elevate plasma catechelamine levels (2) and affect α– and β–adrenoceptor activity (3). Although there are some reports on the effects of hydralazine on sympathatic transmission and release (4-5), its actions on α– and β– adrenoceptors have not been studied precisely. The present investigation was undertaken to study the effects of chronic hydralazine treatment *in vivo* on methoxamine-and isoproterenol-induced responses in cardiac preparations of control and diabetic rats.

METHODS

Animal treatments : Adult female Wistar rats (175-225 g) were made diabetic by an injection of STZ (55 mg/kg) into the tail vein. STZ was dissolved in 0.1 mM citrate buffer (pH 4.5). Control animals

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were injected with a similar volume of the vehicle. On the third day, the animals were checked for the presence of glucosuria using enzymatic test strips. The rats displaying glucosuria greater than 2% were used as the diabetic group. The rats were then randomly divided into four groups : controls; hydralazine-treated controls; diabetics and hydralazine treated diabetics. Hydralazine was added to the drinking water of the treated animals (0.5 to 0.6 mg/ml for contol and 0.08 to 0.1 mg/ml for the diabetic). Hydralazine intake was determined as 1000 × [average fluid intake per day (ml) × concentration of hydralazine (mg/ml)/body weight (g)]. The water intake and the body weight of the animals were monitored and the concentration of hydralazine was adjusted such that the animals drank hydralazine in the dose of 50 ± 2 mg/kg/day. The animals were maintained for six weeks and had access to food and water *ad libitum*. The heart rate and systolic blood pressure of these animals were monitored weekly by the tail-cuff method.

Preparation of isolated tissues: After six weeks the animals were stunned by a sharp blow to the head and killed by decapitation. The blood samples were collected and the serum was separated by centrifugation. The serum was stored at – 20°C until assayed. The hearts were quickly removed and placed in Chenoweth-Koelle (CK) buffer (pH 7.4), which was maintained at 37°C and constantly bubled with carbogen (95% O_2 + 5% CO_2). The composition of CK was (in mM). NaCl, 120; KCl, 5.6; $CaCl_2$, 2.2; $MgCl_2$, 2.1; Glucose, 10.0; $NaHCO_3$, 19.2; EDTA, 0.03.

The right and left atria were quickly dissected out and mounted in organ baths containing CK buffer which was maintained under the conditions mentioned above. Right atrium was allowed to beat spontaneously. Left atria were stimulated with 5 msec square wave pulses of 20 threshold voltage, at 1 Hz using a Grass stimulator, Model SD-9. The responses were recorded using a force displacement transducer which was coupled to a polygraph. A resting tension of 1g was applied to all preparations and they were stabilized for 60 min before exposure to any drug.

The preparations were exposed to the graded doses of methoxamine (1×10^{-8} to 1×10^{-4}M) or isoproterenol (1×10^{-9} to 1×10^{-6}M) added in a cumulative manner. The contact time for each dose of methoxamine was 5 min whereas, that of isoproterenol was 30 sec.

Serum analysis: Serum glucose was estimated by the glucose oxidation method using a peridochrom glucose kit and the total lipid concentration in serum was determined by the sulfophosphovanillin reaction method using a total lipid kit. Both kits were obtained from Boehringer Mannheim. Cholesterol and triglycerides were estimated by an enzymatic-colorimetric method using the respective kits from Boehringer-Mannheim.

Serum immunoreactive insulin was assayed by a radio-immunoassay method using an Amersham insulin RIA kit. The T_3B index and serum T_4 levels were determined by a radio-immunoassay method using the Triobead-125 T_3 uptake kit and Tetrabead-125 kit respectively, obtained from Abbott Laboratories.

Drug used:

Streptozotocin (SIZ), isoproterenol hydrochloride and hydralazine hydrochloride were purchased from Sigma (U.S.A.). Methoxamine hydrochloride was generously provided by Burroughs Wellcome (U.S.A.). All drugs, except SIZ, were dissolved in glass distilled water.

Statistical analysis: The Student's t-test was employed to compare a single experimental group to control. One-way analysis of variance, followed by Neuman Keul's test, was used when comparing three or more groups. The level of significance was set at $P<0.05$.

RESULTS

Injection of SIZ into the animals resulted in a diabetic state. The serum glucose and serum lipid levels were significantly elevated in the diabetic group (Table I). These animals also exhibited a significant loss of body weight, hypoinsulinemia, bradycardia and mild hypertension. Other symptoms normally associated with the diabetic state such as polydipsia, polyuria and polyphagia were also seen. Treatment with hydralazine did not prevent the hyperglycemia, hypoinsulinemia or the loss of body weight. However, it successfully prevented the diabetes-induced bradycardia, hypertension and hyperlipidemia (Table I). Fluid intake was further increased in diabetic rats after hydralazine treatment. Another important finding was the significant decrease

TABLE I: Effect of hydralazine treatment on SIZ diabetic rates.

	Untreated Control n = 5	*Hydralazine-treated Control n = 5*	*Untreated Diabetic n = 6*	*Hydralazine-treated Diabetic n = 5*
Heart rate (b/m)	385.2±12.4	410.0±7.7*	328.8±8.9*	360.0±22.4**
Blood pressure (mmHg)	137.0±4.1	117.5±3.2	173.7±3.7*	140.0±7.3***
Serum glucose (mg/dl)	105.7±19.2	96.6±7.5	441.8±9.7*	431.5±25.7*
Serum insulin (uU/ml)	38.6±9.7	33.0±4.2	14.0±1.7*	12.0±2.0*
Serum total lipids (mg/dl)	424.3±47.2	571.7±68.1	1818.8±225.8*	895.6±85.6**
Serum triglyceride (mg/dl)	113.8±11.4	125.7±15.9	439.8±76.0*	279.2±60.6**
Serum cholesterol (mg/dl)	75.4±10.7	78.3±7.3	168.6±18.0*	114.5±4.5
T_3B index (%)	57.4±1.3	57.7±1.0	46.3±1.5*	54.1±0.8
T_4 value (ug/dl)	4.8±0.3	4.7±0.4	2.5±0.2*	4.0±0.5

* Significantly different from untreated control (P<0.05).
** Significantly different from untreated control and untreated diabetic (P<0.05).
*** Significantly different from untreated diabetic (P<0.05).

in the T_3B index and T_4 levels in the serum of diabetic rats. Hydralazine-treated diabetic rats did not show the decrease in T_3B index or T_4 levels (TableI).

Effect of hydralazine treatment on methoxamine and isoproterenol induced responses: Insoproterenol produced a dose-dependent positive inotropic and positive chronotropic effect in left (Fig.1) and right atria (Fig.2), respectively. The responses to isoproterenol were significantly decreased in the preparations obtained from diabetic animals. The pD_2 value was not altered. However, the maximum response obtained was found to be less in both these preparations (Table II). Left atria obtained from hydralazine-treated animals produced

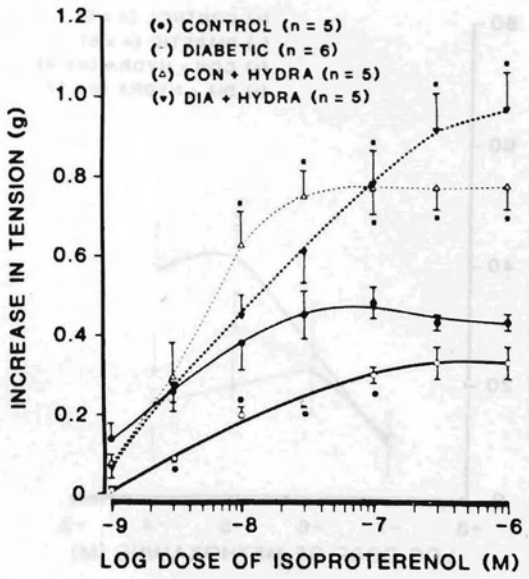

Fig. 1 : The effect of isoproterenol on left atrium of rat. Each point represents the mean and each bar indicates ±SEM of 5–6 experiments. (*) denotes significantly different from untreated control (P<0.05).

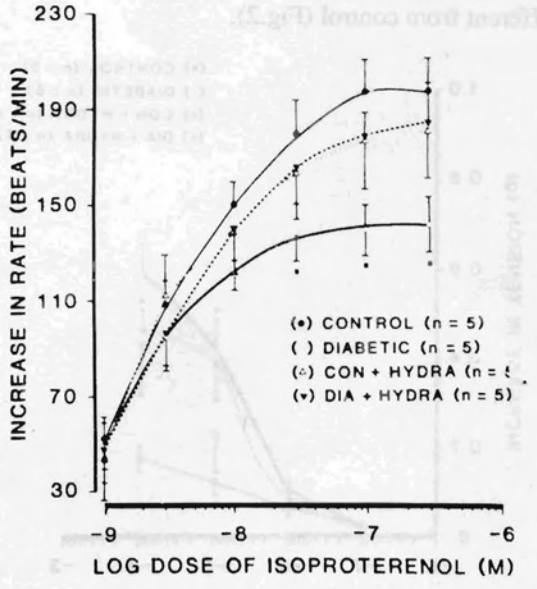

Fig. 2 : The effect of isoproterenol on right atrium of rat. Each point depicts the mean and each bar represents ±SEM of 5–6 experiments. (*) indicates significantly different from untreated control (P<0.05).

TABLE II : pD_2 and maxima for isoproterenol and methoxamine in right and left atrium. Results are expressed as mean ± SEM; n, number of experiments.

		Left atrium		*Right atrium*	
		pD_2	*Maximum tension (g)*	*pD_2*	*Maximum rate (b/m)*
Control-untreated (n = 15)	Isoproterenol	8.32±0.14	0.51±0.04	8.60±0.06	190.00±12.14
	Methoxamine	4.68±0.14	0.17±0.03	6.53±0.18	23.88±3.97
Control hydralazine treated (n = 5)	Isoproterenol	8.49±0.09	0.77±0.06*	8.71±0.16	170.00±19.68
	Methoxamine	5.49±0.18	0.43±0.12*	6.59±0.20	28.75±9.65
Diabetic-untreated (n = 16)	Isoproterenol	8.09±0.10	0.33±0.04*	8.92±0.12	140.83±19.68*
	Methoxamine	5.19±0.11	0.58±0.07*	6.24±0.14	56.25±4.50*
Diabetic-hydralazine treated (n = 15)	Isoproterenol	8.02±0.11	0.95±0.08*	8.68±0.17	180.00±7.90
	Methoxamine	5.36±0.06	0.45±0.05*	6.39±0.32	27.00±6.04

*Significantly different from untreated control (P<0.05).

a greater response to isoproterenol as compared to control (Fig.2). The maximum response was significantly greater in hydralazine-treated control and diabetics as compated to their own controls (Table II). The isoproterenol-induced positive chronotropic response in right atria from hydralazine-treated animals was not significantly different from control (Fig.2).

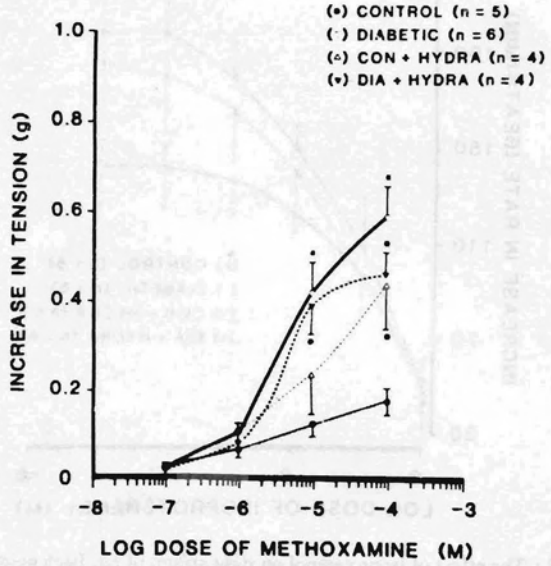

Fig. 3: The effect of methoxamine on left atrium of rat. Each point and bar depicts mean±SEM of 5-6 experiments. (*) denotes significantly different from untreated control (P<0.05).

Like isoproterenol, methoxamine produced a dose dependent increase in tension and rate in left atria (Fig.3) and right atria (Fig.4), respectively. However, unlike isoproterenol, the responses to methoxamine were significantly increased in diabetic preparations. Hydralazine treated left atria also produced a significantly

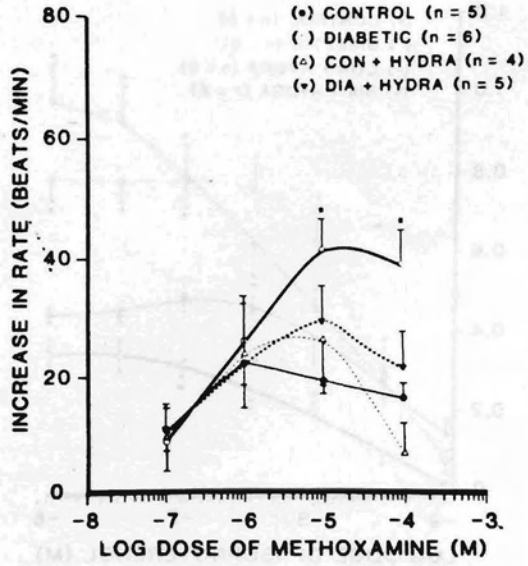

Fig. 4: The effect of methoxamine on right atrium of rat. Each point and bar represents mean±SEM of 5-6 experiments. (*) indicates significantly different from all other groups (P<0.05).

greater positive inotropic response to methoxamine (Fig.3). However, the positive chronotropic effect of methoxamine was not significantly different in right atria treated with hydralazine (Fig.4). The pD_2 value was not changed in either of these preparations (Table II).

DISCUSSION

In the present study STZ diabetes induced bradycardia, hypertension and hyperlipidemia were found to be prevented by hydralazine treatment (Table I). It is possible that hydralazine can prevent the elevation of lipids by improving thyroid function or an action on adrenoceptors, or catecholamine synthesis and release. In the present study, both T_4 and T_3 levels were found to, be decreased in diabetics.

Hydralazine treatment increased both T_3B as well as T_4 levels in diabetic rats (Table I). The mechanism of this action is not understood at present but presumably could be through TSH release and a correction of the T_3 to T_4 conversion deficit.

The positive chronotropic and inotropic effects of isoproterenol in right and left atria were significantly reduced, while those of methoxamine were significantly increased in the preparations obtained from the diabetic rats (Fig.1-4). These results are in agreement with those reported earlier (6). Recently, it has been reported that the increased responsiveness of methoxamine in diabetes could be a result of the associated hypothyroidism, since T_3 treatment of diabetic rats prevented such an increase (7). In the present study it was found that hydralazine treatment also prevented the increased responsiveness to methoxamine in right atrium of diabetic rats (Fig.4). Hydralazine was also found to correct the state of hypothyroidism. Thus, it is possible that hydralazine-induced changes in the responsiveness of right atria to methoxamine could be through its effects on thyroid function.

The decrease in β-adrenoceptors may be due to a "down-regulation" mechanism since elevated plasma catecholamine levels have been reported in diabetic animals (8).

Hydralazine significantly enhanced the responses to isoproterenol in left atrium in both control and diabetic rats (Fig.2). The responses to isoproterenol were not depressed in diabetic right atrium treated with hydralazine. It is yet to be determined if hydralazine can decrease catecholamine levels in control or diabetic rats.

Diabetic animals have an increased plasma lipid content and alterations in dietary lipids have the capacity to affect β-adrenoceptor density in the heart (9). Diabetes-induced metabolic derangements may be responsible for altered receptor characteristics in diabetic rats. In the present study lipid levels were found to be increased in diabetics and the increases were prevented by hydralazine. Thus, another mechanism for hydralazine-induced improvement in cardiac function and alteration in responsiveness to adrenoceptor agonists may be through its effects on lipid content.

Besides the decrease of β-adrenoceptors in diabetics, there is a possibility of an alteration in post-receptor mechanism(s) responsible for the decrease in responsiveness to isoproterenol in right and left atrium such as stimulation of adenylate cAMP (10), phosphorylase activation (11) and Ca^{++} uptake by sarcoplasmic reticulum (12). Hydralazine is known to affect Ca^{2+} fluxes. However, the role of Ca^{2+} on the hydralazine effects in the diabetic animal is purely speculative at this time.

In conclusion, hydralazine-induced alterations in the responsiveness to α-adrenoceptor agonist could partly be due to its ability to prevent diabetes-induced hypothyroidism. The reduction of isoproterenol-induced responses in diabetic preparations and an increased responsiveness to isoproterenol after hydralazine treatment seem to be effects that are independent of hypothyroidism. Post-receptor changes and metabolic derangements may be responsible for these effects.

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